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14. ABSTRACT

This report summarizes the efforts of our team consisting of researchers from Rice University and M. D. Anderson Cancer Center, in the DoD funded CDMRP award towards the development of an integrated nano particle based imaging and therapy of breast cancer. We have used both gold nano particles (Au-NPs) and gold nanoshells for specifically targeting cancer cells. This has been accomplished in two ways. First by attaching anti-human epidermal growth factor receptor 2 (anti-HER 2) antibodies to gold nanoshells for targeting HER 2 expressing SK-BR3 breast carcinoma cells. The second approach is by building biologically active networks of Au-NP and bacteriophages which can be used as in vivo biological sensors for non invasive optical molecular fingerprinting, and cell targeting agents.

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Introduction

Gold nanoshells are are a new class of nanoparticles with a highly tunable surface plasmon resonance. They consist of a spherical silica core covered with a thin shell of gold. The surface plasmon resonance of these nanoshells can be changed by changing the size of the core silica particles and the thickness of the gold shell. The Halas nanoengineering group at Rice University was the first to develop these particles[1] and demonstrate their application to photothermal ablation of tumors.[2] The current DoD funded CDMRP award is for the development of nanoparticle based methods for detection, imaging and therapy of breast cancer.

This report summarizes the efforts of our team consisting of researchers from Rice University and M. D. Anderson Cancer Center, in the DoD funded CDMRP award towards the development of an integrated nano particle based imaging and therapy of breast cancer. We have used both gold nano particles (Au-NPs) and gold nanoshells for specifically targeting cancer cells. This has been accomplished in two ways. First by attaching anti-human epidermal growth factor receptor 2 (anti-HER 2) antibodies to gold nanoshells for targeting HER 2 expressing SK-BR3 breast carcinoma cells. The second approach is by building biologically active networks of Au-NP and bacteriophages which can be used as in vivo biological sensors for non invasive optical molecular fingerprinting, and cell targeting agents.

Body

PROJECT 1: Bioconjugation-based Targeting of Nanoparticles for imaging and therapy:

Task 1: Identification of optimal ligand-receptor pair for vascular targeted therapy in breast cancer (months 1-36).

Accomplishment: In continuation of our previous studies, we have demonstrated that anti-human epidermal growth factor receptor 2 (anti-HER 2) can be consistently bound to gold nanoshells via a bi functional poly ethylene glycol (PEG) linker to form immunonanoshells. We have now quantified the density of this antibody attached to the immunonanshell surface by an ELISA style assay. This is confirmed by TEM imaging of gold labeled antibodies attached to the immunonanoshells (Figure 1) Antibody densities of ~ 150 antibodies/nanoshells have been achieved.[3]

Filamentous bacteriophage (phage) can be easily engineered genetically to display ligand proteins that will bind to selected tissue. Integrating phage display technology with gold nanoparticles allows for targeting and optical imaging of specific tissue. We have investigated Au-phage networks for imaging and molecular finger printing of tumor cells, both in vitro and in vivo in tumor bearing mice.[4, 5]

Task 2: Development of bioconjugate chemistry for binding targeting peptides and antibodies to nanoshells and nanoemitters (months 12-48).

Accomplishment: The gold nanoparticle (Au-NP)-phage and Au-NP-phage-imidazole networks used for image contrast enhancement and non invasive optical detection were synthesized as follows: Au nanoparticales were prepared by the standard citrate reduction of

gold(III) chloride (99.99+% from Aldrich). Samples consisted of 500 μL of Nanopure water, 500 μL of 100 mM borate buffer at pH 8.0, 3 μL of imidazole solution at 99.5% (Fluka), with 500μL of 0.32 nM Au-NP solution added last. To ensure that the system had reached equilibrium, all measurements were made 12 h after sample preparation. Samples were vigorously mixed to guarantee homogeneity before each measurement. Imidazole solutions consisted of separate dilutions (in Nanopure water) ranging from 0.24 to 500 μM were investigated.[5]

PROJECT 2: Nanoparticle-based Image Enhancement:

Task 1: Investigation of the effectiveness of gold nanoshells and rare earth nanoemitters as image enhancers in tomographic infrared imaging, (months 1-24).
Accomplishments: We have studied the optical behaviour of gold nanoshells embedded in tissue. Using gold nanoshells of different sizes and optical parameters we have employed Monte-Carlo models to predict the effect of varying concentration of the different nanoshells on tissue reflectance.[6] These studies will be used to optimize the dosage of nanoshells required for imaging and therapy.

Task 2: Evaluation of the improvements in image contrast and resolution due to nanoshell-based and nanoemitter-based contrast enhancers, (months 12-36).
Work is ongoing on task 2.

Task 3: Demonstration of feasibility of targeted nanoshell-based imaging and nanoemitter-based imaging in a mouse tumor model, (months 12-48).
Accomplishments: We have investigated biologically active networks of gold nanoparticles-bacteriophage-imidazole as labels for non invasive optical detection of specific tumors. Spontaneous aggregation of Au-NPs on phage occurs without genetic modification of the pVIII major capsid proteins. The Au-NP- phage network depends upon the concentration of phage and can be further modified by the presence of imidazole. The aggregation of the gold particles within the Au-NP-phage network broadens and red-shifts the surface plasmon resonance of the gold particles to the biologically accessible water window where light penetration through tissue is optimal. These biologically active networks effectively integrate the signal reporting properties of gold NPs, while preserving the biological activity of the phage. Melanoma cells that express high levels of α_v-integrins were chosen to demonstrate the immunofluorescence staining by the Au-phage network. The cell surface receptor is well characterized for displaying the peptide CDCRGD-CFC (RGD-4C) Figure 2 (supporting data) shows the results of the immunofluorescence based phage binding assay of Au-RGD-4C networks.
When examined by confocal microscopy, Au–phage networks could serve as sensitive reporters to localize and evaluate ligand binding and receptor-mediated internalization events (Figure 3 supporting data). The differences in the structure of the targeting networks result in distinct kinetics of the internalization event that follows ligand–receptor binding. By incorporating imididazole into the nanoarchitecture of the networks, we were able to influence the localization of the Au–phage networks to either the cell surface or cytoplasm. More compact networks with a higher fractal dimension (Au–RGD-4C-imid) preferentially

localized at the cell surface, whereas those with a lower fractal dimension (Au–RGD-4C) were internalized (Figure 3).[4, 5]

PROJECT 3: Molecular Fingerprinting

Task 1: Examination of the feasibility of noninvasive "molecular fingerprinting" in a tissue culture model, (months 1-24).
Accomplishments: We have also demonstrated the use of biologically active networks of Au NP-phage as optically active labels for non invasive detection using surface enhanced Raman spectroscopy (SERS). [4, 5]

Task 2: Investigation of spectroscopic detection of early lesions, (months 12-48).
Accomplishments: Acidic pH is a common characteristic of human tumors.[7] Normal cells have a pH of 7.4 whereas most breast cancer tumors have a pH around 6.8. We have developed a nanoshell based pH sensor that can be used to monitor the pH in vivo to determine carcinogenicity. The Au nanoshell with a pH-sensitive molecular adsorbate (para-mercapto benzoic acid in this case) functions as a standalone, all-optical nanoscale pH meter that monitors its local environment through the pH-dependent surface-enhanced Raman scattering (SERS) spectra of the adsorbate molecules. By using a statistical learning theory analysis of the SERS spectra, a quantitative pH sensor has been developed. The average accuracy of the nano pH-meter was found to be ±0.10 pH units across its operating range.[8]

PROJECT 4: Nanoshell-based Photothermal Cancer Therapy

Task 1: Evaluation of the therapeutic effect of targeted nanoshells in animal models of breast cancer, (months 1-36).
Accomplishments: Work is in progress on task 1.

Task 2: Development of a strategy for combined imaging and therapy, (months 12-48).
Accomplishments: The addition of a tumor-specific antibody should increase the specificity of the nanoshell therapy, as indicated by the initial in vitro results.[9] Anti HER2 immunonanoshells bind to targeted cells whether the cells are alone or adjacent to healthy cells. Upon laser irradiation, the cells bound by nanoshells are killed, leaving the healthy cells unharmed (See Figure 4) .[3] Successful treatments not only require the laser and nanoshells to be present simultaneously, but in order for nanoshells to be present in vitro, they must actively bind to the targeted cell type. In vivo, the accumulation of immunonanoshells within a tumor will not simply rely on the enhanced permeability of tumors as previously demonstrated.[10] In addition to utilizing the elevated permeability of tumors, the ability of immunonanoshells to bind tumor cells should further promote the localization of nanoshells within the tumor. The increased specificity by the antibodies has tailored the immunonanoshell for higher accumulations at targeted tissues and thus improved the nanoshell therapeutic efficiency.

Key Research Accomplishments

- HER2 immunonanoshells were shown to specifically target SK BR3 in both imaging and photothermal ablation studies without destroying neighboring healthy cells.

- Au-phage and Au-phage-imidazole networks can be engineered to target specific tissue types. These networks have been shown to improve imaging and molecular fingerprinting of tumor sites in both in vitro and in vivo studies.

- We have developed an all optical nanoshells based pH sensor with a sensitivity of 0.1 pH units. This will allow distinction of cancerous, precancerous and normal cells.

- We are developing mathematical Monte Carlo models to optimize the nanoshells concentration required for imaging

Reportable Outcomes

Published

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Book Chapters

1. West, J. L.; Drezek, R. A.; Halas, N. J., Nanotechnology Provides New Tools for Biomedical Optics. In Chapter 25, CRC press: 2006; pp 1-24.

In Press

1. Fu, K., Sun, J., Lin, A., Wang, H., Halas, N., and Drezek R. Polarized Angular Dependent Light Scattering Properties of Bare and PEGylated Gold Nanoshells. Current Nanoscience. (2006).

<u>Conference Proceedings</u>

Presentations

2. Loo, C., West, J., Halas, N., and Drezek, R. Nanoshells for Real-Time NIR RCM-Based Molecular Imaging. Biomedical Engineering Society Annual Meeting. Baltimore, MD. October 2005.

3. Loo, C., West, J., Halas, N., and Drezek R. Nanoshell-Based Molecular Imaging of Breast Tissue. MSTP Annual Symposium. Houston, TX. 2005.

4. Lowery A., Gobin A.M., Day E., Shah K., Halas N., Drezek R., West J. "Antibody-Conjugated Metal Nanoshells for Tumor Specific Photothermal Therapy" Society for Biomaterials, Pittsburgh, PA, April 2006

5. Lowery A., Loo C., O'Neal P., Hirsch L., Halas N., Drezek R., West J. "Antibody-Conjugated Metal Nanoshells for Tumor Specific Photothermal Therapy" Society For Biomaterials, Memphis, TN, April 2005
6. Lowery A., O'Neal P., Hirsch L., Halas N., Drezek R., West J."Antibody-conjugated Metal Nanoshells for Selective Photothermal Therapy" Rice University Institute of Biosciences and Bioengineering Graduate Training Programs Annual Retreat. Rice University, Houston, TX, March 2005

7. Lowery A., Loo C., O'Neal P., Hirsch L., Halas N., Drezek R., West J."Antibody-conjugated Metal Nanoshells for Selective Photothermal Therapy" Houston Society for Engineering in Medicine. University of Houston, Houston, TX, Feb 2005

Poster Presentations

1. Lowery A., Gobin A.M., Day E., Shah K., Halas N., West J. "Immunonanoshells for Selective Photothermal Therapy" American Association of Cancer Research: Molecular Targets and Cancer Theraputics, Philadelphia, PA, Nov. 2005

2. Lowery A., Gobin A.M., Day E., Shah K., Halas N., West J. "Immunonanoshells for Selective Photothermal Therapy" (poster) Nanotech Initiative. Rice University. Houston, TX, Oct. 2005

Related Invited Talks

1. Drezek, R., West, J., and Halas, N. Nanoshells for Imaging and Therapy of Breast Cancer. DOD Era of Hope Meeting. 2006.

2. Design and Implementation of a Standalone Raman-based All-optical Nanosensor", Quantum Electronics and Laser Science Conference (QELS), Baltimore, May 2005.

3. "Nanoshells: applying Nanotechnology to harvest light for biomedicine" at "Minds without Borders: Frontiers in Medical Research", Medical Sciences Graduate Student Association, University of Calgary, Calgary, Alberta, Canada, May 2005.

4. "Nanoshells: Seamless Integration of Cancer Imaging and Therapy", Era of Hope Conference, Philadelphia, PA, June 2005.

5. "Nanoshells: using Nanotechnology to harvest light for biomedicine", The 2005 Landsdowne Lecturer in Chemistry and Electrical Engineering, University of Victoria, CA, September, 2005 (popular lecture)

6. "Nanoshells: from plasmon physics to cancer therapy", Distinguished Speaker Series, Center for Nano and Molecular Science and Technology, University of Texas, February 2005.

7. " Nanoshells: from Plasmon physics to cancer therapy", Invited speaker series, Department of Chemistry, University of Utah, February 13, 2006.

8. "Designing Nanotools for Biomedicine", 28th Annual Symposium of the Burnham Institute, La Jolla, CA, April 2006.

9. "Designing Optical Nanotools for Biomedicine", The Dorothy J. Killam Lecture, Montreal Neurological Society, McGill University, May 2006.

10. " Nanoshells: from plasmon physics to cancer therapy" and "Nanoengineered Plasmonic substrates for surface enhanced spectroscopies", Colloquium and Tutorial, Kavli Nanoscience Institute Distinguished Speaker Series, Caltech, May 2006.

11. "Beyond Drugs, Cancer, and Fear: The promise of nanotechnology in biomedicine", Perspectives on the Future of Science and Technology Conference, U. S. Department of State sponsorship, Lake Como, IT, May 2006.

12. "Taking the (Nano) device approach: applications of nanotechnology in the diagnosis and treatment of cancer and other diseases," Gordon Research Conference in Molecular Therapeutics of Cancer, Oxford, UK, July 2006.

13. "An all-optical SERS-based pH nanosensor," SPIE (Society for Photo-optical Instrumentation Engineers) Annual Meeting, San Diego, CA, August 2006.

Conclusions

We continue to make significant progress in all our goals. This year the focus of this synergistic team of scientists at Rice University and M. D. Anderson has been on specific targeting of the breast carcinoma cell. We have made significant progress in developing HER2 immunonanoshells for specifically targeting SK-BR3 adeno-carcinoma cells with optically active nanoshells. This has been demonstrated to be specific and useful in both signal enhancement for imaging and photothermal ablation of the cancer cells. We have also approached targeting of cancer cells by engineering phage display peptides to target specific cancer cells. By creating biologically active networks of gold colloid nanoparticles and targeting phage we have achieved integration of imaging tumors with molecular fingerprinting of these tumors. This has been demonstrated both in vitro and in vivo mouse tumors.
We have used Monte-carlo models to simulate the reflectance of nanoshells embedded in tissue. Working with a variety of nanoshells of different sizes and optical properties, we have tried to optimize the nanoshells for both imaging and photothermal ablation. The current results encourage future work in integrating imaging and therapy into a single non invasive technique which can be carried out with minimal discomfort to the patient.

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Supporting Data

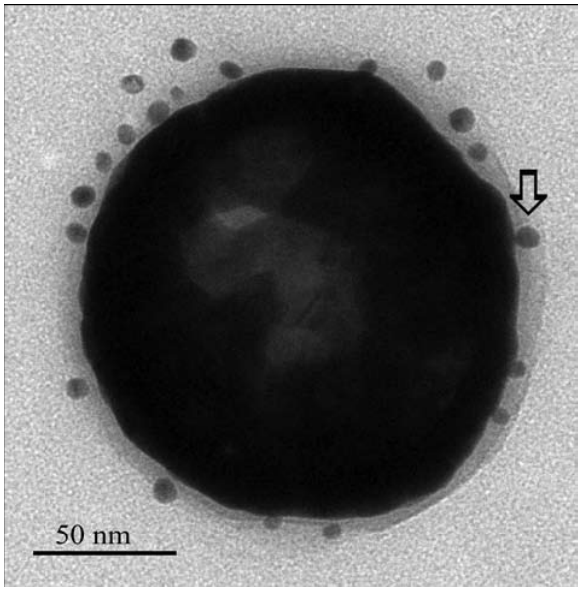

Figure 1: Transmission electron micrograph of an immunonanoshell with gold labeled antibodies (example indicated by arrow) shows several antibodies bound to the nanoshell surface within the hazy polyethylene glycol (PEG) layer.

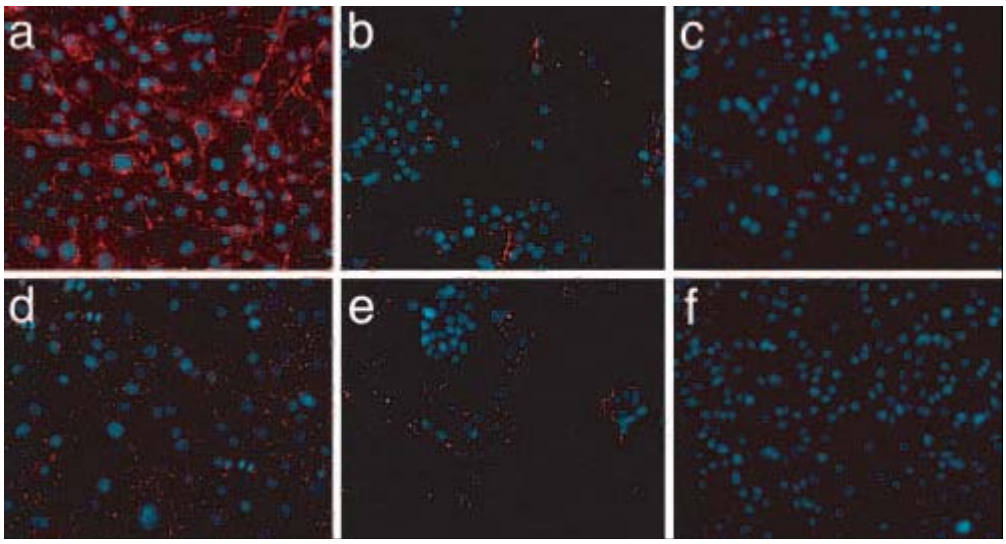

Figure 2: Immunofluorescence-based phage binding and internalization assay with cultured melanoma cells. The red color represents fluorescence related to the RGD-4C peptide, and the blue color shows fluorescence of DAPI-stained cell nuclei. Cells were incubated with different phage preparations, all carrying a phage input of 1.0 $x10^{7}$ TU. (*a*) Au–RGD-4C-displaying phage. (*b*) Cells preincubated with the RGD-4C synthetic peptide (1.0 $x10^{-3}$ nM for 30 min) followed by addition of Au–RGD-4C. (*c*) RGD-4C phage (no Au). (*d*) Au–fd-tet (negative control). (*e*) Cells preincubated with the RGD-4C synthetic peptide (1.0 $x10^{-3}$nM) followed by addition of Au–fd-tet (negative control). (*f*) fd-tet phage (negative control).

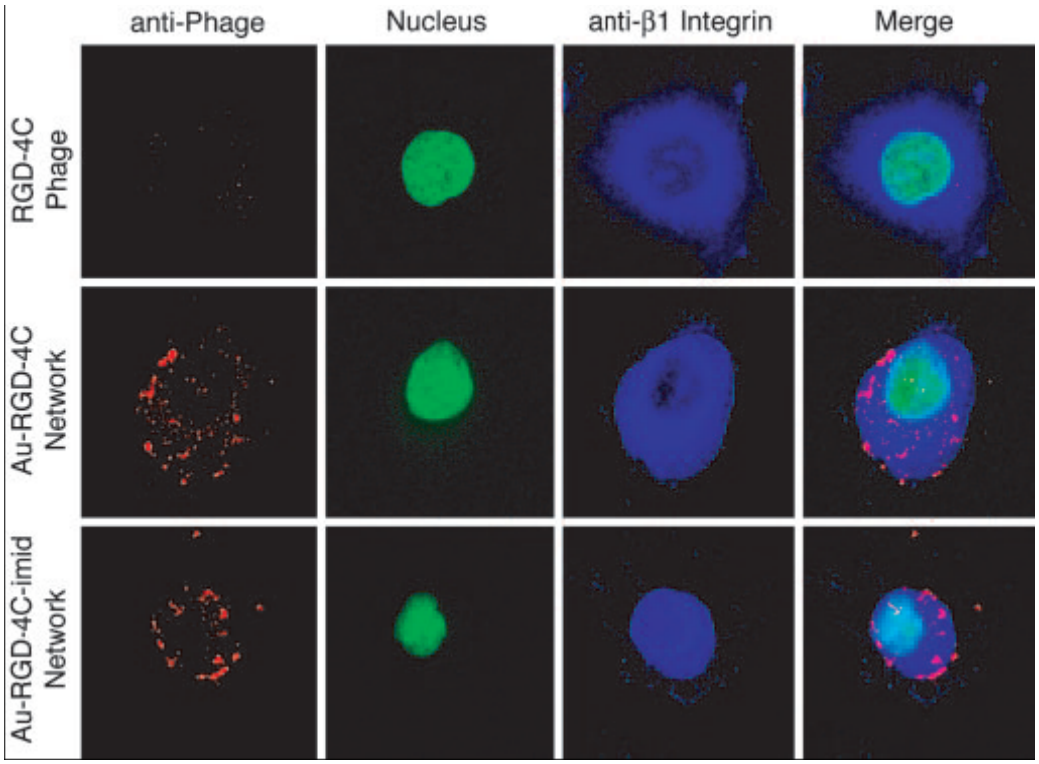

FIGURE 3: Confocal fluorescence. Shown are KS1767 cells incubated with phage preparations (input of 1.0 _ 107 TU) and labeled with anti-fd bacteriophage antibody (red, first column), SYTOX green nucleic acid stain (green, second column), and an anti-β_1-integrin antibody demarking the cell surface (blue, third column). The fourth column shows merged images: RGD-4C phage, Au–RGD-4C networks, and Au–RGD-4C-imid networks (controls for each of the respective RGD-4C phage preparations are shown)

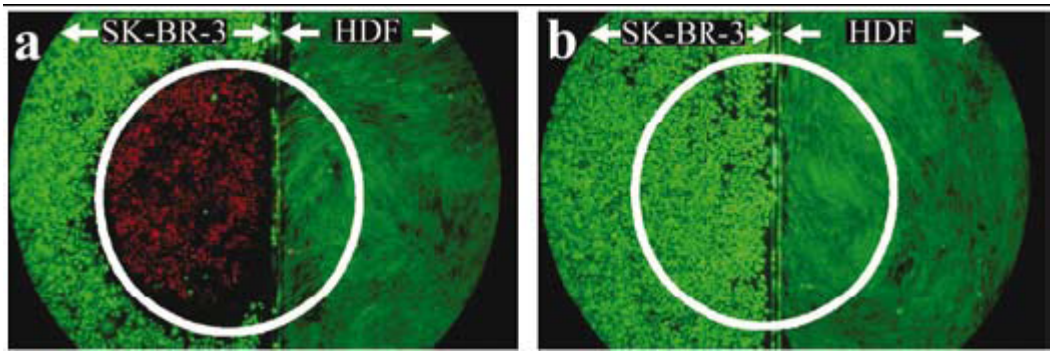

Figure 4: Two cell types, SK-BR-3 (left side of a and b) and HDF (right side of a and b) cells, were grown on glass cover slip and aligned as shown prior to the experiment.
(a) Anti-HER2 immunonanoshells bound to the HER2 expressing SK-BR-3 cells resulted in targeted cell death after laser irradiation. The laser area is outlined in white.
(b) Nanoshells coated in PEG only did not bind cells and laser irradiation produced no area of cell death.